

CLAIMS

What is claimed is:

1. A high growth methanotrophic bacterial strain which:
  - (a) grows on a C1 carbon substrate selected from the group consisting of methane and methanol; and
  - (b) comprises a functional Embden-Meyerhof carbon pathway, said pathway comprising a gene encoding a pyrophosphate dependent phosphofructokinase enzyme, the gene selected from the group consisting of:
    - (a) an isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO:6;
    - (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS;
    - (c) an isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 437 amino acids that has at least 63% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:6; and
    - (d) an isolated nucleic acid molecule that is complementary to (a), (b) or (c).
2. A high growth methanotrophic bacterial strain according to Claim 1 wherein the strain optionally contains a functional Entner-Doudoroff carbon pathway.
3. A bacterial strain according to Claim 1 having at least one gene encoding a fructose biphosphate aldolase enzyme.
4. A bacterial strain according to Claim 3 wherein at least one gene encodes a fructose biphosphate aldolase enzyme having the amino acid sequence selected from the group consisting of SEQ ID NO:16 and SEQ ID NO:18.
5. A bacterial strain according to Claim 2 having at least one gene encoding a keto-deoxy phosphogluconate aldolase.
6. A bacterial strain according to Claim 5 wherein at least one gene encodes a keto-deoxy phosphogluconate aldolase enzyme is selected from the group consisting of:

- (a) an isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO:20;
- (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS;
- (c) an isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 212 amino acids that has at least 59% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:20; and
- (d) an isolated nucleic acid molecule that is complementary to (a), (b) or (c).
7. A bacterial strain according to any of Claims 1 or 2 having a gene encoding a polypeptide involved in carbon flux wherein the polypeptide is selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18 and 20.
8. A bacterial strain according to any of Claims 1 or 2 optionally comprising a denitrifying enzymatic pathway.
9. The bacterial strain of Claim 8 wherein the enzymes of the denitrifying pathway are polypeptides having the amino acid sequences selected from the group consisting of SEQ ID NO:40, 42, 44, 46, 48, 50, 52, 54, 56, 58 and 60.
10. The bacterial strain of any of Claims 1 or 2 having genes encoding exopolysaccharide synthesizing enzymes, the enzymes selected from the group consisting of SEQ ID NO:22, 24, 26, 28, 30, 32, 34, 36, and 38.
11. The bacterial strain of any of Claims 1 or 2 having genes encoding isoprenoid synthesizing enzymes, the enzymes selected from the group consisting of SEQ ID NO:62, 64, 66, 68, 70, 72, 74, 86, and 78.
12. The bacterial strain of Claim 1 wherein the strain is a *Methylomonas* sp.
13. The bacterial strain of Claim 12 having a 16s RNA profile as set forth in SEQ ID NO:81.
14. The bacterial strain of Claim 1 wherein, when the C1 carbon substrate is methanol, the strain produces glycogen comprising at least about 50 % dry weight of biomass.

15 The bacterial strain of either Claim 1 or Claim 14 wherein the methanol concentration in the medium is about 2.5% (vol/vol).

16. The bacterial strain of any of Claims 1 or 2 having a yield of greater than 1.0 grams of cell mass per gram of methane consumed.

5 17. The bacterial strain of any of Claims 1 or 2 having a yield of greater than 0.5 grams of cell mass per gram of methane consumed.

18. The bacterial strain of any of Claims 1 or 2 having a carbon conversion efficiency of greater than 40 g/mol methane/g/ mol biomass.

10 19. The bacterial strain of any of Claims 1 or 2 having a carbon conversion efficiency of greater than 65 g/mol methane/g/ mol biomass.

20. The bacterial strain of any of Claims 1 or 2 having a carbon conversion efficiency of greater than 70 g/mol methane/g/ mol biomass.

21. A high growth methanotrophic bacterial strain which grows on a C1 carbon substrate selected from the group consisting of methanol and methane, comprising the 16s RNA sequence as set forth in SEQ ID NO:81 and having at least one gene encoding a pyrophosphate dependent Phosphofructokinase enzyme.

22. A high growth methanotrophic bacterial strain according to Claim 20 optionally having at least one gene encoding a keto-deoxy phosphogluconate aldolase.

23. A high growth methanotrophic bacterial strain having the ATCC designation PTA 2402.

24. A method for the production of single cell protein comprising:

25 a) contacting the bacterial strains of any of the Claims 1, 2, 3, 5, or 18 with C1 carbon substrate, selected from the group consisting of methane and methanol, in a suitable medium for a time sufficient to permit the expression and accumulation of single cell protein; and

b) optionally recovering the single cell protein.

30 25. The method of Claim 23 wherein the C1 carbon substrate is contacted with the bacterial strain under anaerobic conditions.

26. The method of Claim 23 wherein the C1 carbon substrate is contacted with the bacterial strain under aerobic conditions.

35 27. A method for the biotransformation of a nitrogen containing compound selected from the group consisting of ammonia, nitrate, nitrite, and dinitrogen, comprising contacting the bacterial strain of any of the Claims 8 or 9 with a C1 carbon substrate selected from the group consisting of methane or methanol, in the presence of the nitrogen

containing compound, in a suitable medium for a time sufficient to permit the biotransformation of the nitrogen containing compound.

28. A method for the production of a feed product comprising protein, carbohydrates and pigment comprising the steps of:

- 5                   a) contacting the bacterial strain of any of Claims 1, 2, 3, 5 or 18 with a C1 carbon substrate in a suitable medium for a time sufficient to permit the expression and accumulation of the feed product; and
- b) optionally recovering the feed product.

10           29. A method according to Claim 28 wherein the relative compositions of protein, carbohydrate and pigment are altered through the up-regulation or down-regulation of any one of the genes encoding the proteins selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 15 53, 55, 57, 59, 61, 63, 65, 67, and 69.

30. A method of identifying the high growth methanotrophic bacterial strain of Claim 1 comprising:

- 20                   (a) growing a sample suspected of containing a high growth methanotrophic bacterial strain on a suitable growth medium in the presence of methane as a sole carbon source;
- (b) identifying colonies that grow on the conditions of step (a);
- (c) analyzing the colonies identified in step (b) for the presence of pyrophosphate dependent 25 phosphofructokinase activity.

31. A method according to Claim 30 wherein the colonies of step (b) are additionally analyzed for the presence of a gene selected from the group consisting of:

- 30                   (a) an isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO:6;
- (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS;
- 35                   (c) an isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 437 amino acids that has at least 63% identity based on the Smith-Waterman method of alignment when

compared to a polypeptide having the sequence as set forth in SEQ ID NO:6; and

- (d) an isolated nucleic acid molecule that is complementary to (a), (b) or (c).